

**2007 Environmental and Subsurface Science Symposium,
featuring Biotechnology and Bioremediation**

Poster Abstracts

1. BIODEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE PRESENCE OF PENTACHLOROPHENOL

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A Superfund Site in Libby, MT has demonstrated biodegradation of polycyclic aromatic hydrocarbons (PAHs) in the presence of pentachlorophenol (PCP) in contaminated soil and ground water. PAHs are known to have toxic, mutagenic, and carcinogenic properties; PCP has been used as a bactericide, fungicide, and insecticide, and is a potential human carcinogen. At Utah State University three microorganisms that are characterized as Mycobacteria strains were isolated from the contaminated soil in prepared-bed bioreactors and were demonstrated to degrade PAHs. Genomic sequencing of the three mycobacteria isolates was performed by the U.S. DOE/Joint Genome Institute. Analysis of the sequences showed genes encoding enzymes responsible for the initial steps in biodegradation of PAHs and potential enzymes responsible for degrading PCP. Contaminated ground water at the Libby, MT site is currently treated using two continuous flow bioreactors. Polymerase chain reaction (PCR) analysis showed that sequences specific to the PAH-degrading mycobacteria strains were found in samples from the bioreactors. Although the mycobacteria did not mineralize PCP during the time duration of the experimental tests, the isolates appear capable of mineralizing PAHs in the presence of PCP at the Superfund site in both soil and groundwater environments.

2. SELECTIVE GRAZING OF MICROBIAL MATS BY DIPTERAN LARVAE LEADS TO BIOMAGNIFICATION OF METHYLMERCURY IN A GEOTHERMAL FOOD WEB

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Chemolithoautotrophic and phototrophic microorganisms constitute the base of complex geothermal food webs by fixing carbon for heterotrophic consumers such as invertebrate grazers. While the transfer of carbon between autotrophic producers and microbial consumers inhabiting geothermal systems have been characterized, less is known on the transfer of carbon and toxic material between autotrophic producers and invertebrate consumers. We have observed high densities of stratiomyid larvae associated with autotrophic microbial mats in numerous acid-sulfate-chloride springs (ACS) in Yellowstone National Park (YNP). Elevated levels of total mercury (THg) have been reported in the water of thermal springs in areas of YNP with THg-enriched soil, and THg and mono-methylated mercury (MeHg) have been detected in the mat microbial biomass.

Previous studies have determined that elevated THg in aquatic water quantitatively induces microbial MeHg-demethylating genes, leading to lower MeHg to THg ratios in the aqueous phase. Here we demonstrate that elevated THg not only decreases the MeHg to THg ratio in acid geothermal spring water, but also leads to decreased MeHg to THg ratios in microbial mat biomass which inhabit these springs. In addition, DNA-based methods were employed to demonstrate that stratiomyid larvae selectively graze algal populations of the microbial mat community. Our results suggest MeHg biomagnification in the tissues of stratiomyid larvae as a consequence of grazing microbial mat populations. Similarly, MeHg biomagnification was observed in an avian species observed preying upon stratiomyid larvae inhabiting acid geothermal springs.

Collectively, these findings suggest invertebrate grazing of microbial mat populations serves as a mechanism for MeHg biomagnification in geothermal food webs. In addition, the current analysis alludes to the importance of microbial activity in controlling MeHg biomagnification in higher trophic structures in aquatic-based food webs.

3. STRUCTURAL ROLE FOR FLAGELLA IN BIOFILM FORMATION IN *desulfovibrio vulgaris* HILDENBOROUGH

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Studies on sulfate reducing bacteria (SRB) have been of interest due reduction capabilities during metal corrosion and bioremediation. Sulfate-reducing bacteria are known to grow as biofilms on different surfaces; however, little is known about biofilm growth in SRBs. *Desulfovibrio vulgaris* Hildenborough has been a model organism for SRBs but little research has been conducted on biofilm formation or maintenance. *D. vulgaris* ATCC 29579 (wild-type) and three mutants, $\Delta flaG$, $\Delta fliA$, and ΔMP (lacking the 200kb plasmid) were grown in batch mode in a defined medium with lactate and sulfate and biofilms were allowed to form on glass slides. Wild-type cells were motile and formed a continuous mono-layer of cells on the glass as observed via crystal violet staining and SEM. Initial results indicated that $\Delta flaG$ mutants were motile, while the ΔMP and $\Delta fliA$ mutants were less motile or not motile. Significant amounts of carbohydrate were not measured within wild-type biofilms (0.01 ug hexose sugar per ug protein), and biofilms stained with calcofluor white, Concanavalin A, and congo red revealed little external carbohydrate (e.g. EPS). TEM analysis of wild-type biofilms grown on SiO₂ grids also showed little EPS, but the presence of 'filaments' were observed in both TEM and SEM images. The filaments, possibly a form of modified flagella, were present within wild-type biofilms but fewer were seen in $\Delta flaG$, and were almost completely lacking in the $\Delta fliA$ and ΔMP mutants. Crystal violet staining revealed that $\Delta flaG$, $\Delta fliA$, and ΔMP mutants produced 5-fold, 2-fold, and 3-fold less biofilm compared to the wild-type, respectively. As observed with wild-type biofilms, negligible amounts of carbohydrate were detected within the mutant biofilms. Filtrate samples of the wild-type biofilms were also analyzed and a 1D protein gel indicated that the biofilm matrix was enriched for certain polypeptides. These results indicated that *D. vulgaris* appears to rely on a proteinaceous material to form and maintain its biofilm matrix and flagella, or a modified form of flagella, play an important role in not only initial formation of *D. vulgaris* biofilm but also in biofilm stability.

4. MOLECULAR TOOLS FOR DETERMINING MICROBIAL COMMUNITY CHARACTERIZATION AT TCE CONTAMINATED SITES

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The use of molecular biological tools (MBTs) in environmental engineering is becoming more and more important in research as well as in site assessment and performance monitoring at many remediation sites. MBTs have many advantages over more traditional microbial characterization techniques, namely that the use of DNA doesn't require culture for analysis.

Hill Air Force Base (HAFB) in Ogden, UT has several trichloroethylene (TCE) contaminated groundwater plumes in which a variety of remediation technologies are currently being employed (i.e., pump and treat, soil vapor extraction, bioaugmentation). MBTs such as polymerase chain reaction (PCR), real-time PCR, and automated ribosomal intergenic spacer analysis (ARISA) are currently being performed at the Utah Water Research Laboratory (UWRL) in order to help better characterize the microbial population at several of these sites. Through this microbial characterization the potential for TCE degradation can be

better estimated and changes in microbial population that occur in response to a remediation technique can be monitored over time.

The MBTs currently being used at the UWRL use DNA extracted from soil and/or groundwater from the contaminated plumes at HAFB to determine the presence and quantity of certain types of important microorganisms and key genes, as well as to characterize the microbial population as a whole. Two main groups of microorganisms important to the degradation of TCE are focused on with these MBTs. The first of these groups consists of TCE degrading bacteria including several strains of *Dehalococcoides* as well as those genes responsible for TCE degradation in the bacterial genome such as *vcrA* and *tceA*. The second group of microorganisms consists of iron reducing bacteria such as *Rhodoferrax*, *Geobacter*, and *Shewanella*, which are prominent in the soils around HAFB and compete for electron donor with TCE degraders. Other microorganisms that are also of interest and are being studied include sulfate reducing bacteria and methanogens. Total populations of eubacteria and archaea are also being determined in the characterization of microbial communities at these sites.

This presentation summarizes some of the results of the research using MBTs that is currently being performed at the UWRL. Results from PCR, real-time PCR, and ARISA are shown and compared for samples from HAFB. Methods for achieving enhanced results from environmental samples using MBTs are discussed. Interpretations of the results as well as some of the advantages and disadvantages of using these technologies are also included.

5. MICROBIAL BIODIVERSITY OF GREAT SALT LAKE, UT

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The Great Salt Lake (GSL) in Utah is the largest salt lake in the Americas, the second most saline lake on earth, and the fourth largest terminal lake in the world. Because of its high salinity, the GSL represents unique environmental challenges, and has limited biological diversity with bacteria, algae, diatoms, brine shrimp, and brine flies being the only organisms present in the main (hypersaline) portions of the lake. The study of the lake environment is complicated by the arid climate in the region and the variable yearly precipitation that can highly influence the lake size and salinity. A railroad causeway constructed in the 1950's that divided the lake into essentially two lakes with different salinities, up to 12% in the south arm and 25-30% in the north arm, further complicates the environment. The GSL has a significant role in the ecology and economic development of the surrounding areas. The lake is an important stopping and feeding point for a large numbers of migratory birds following two of the major North American migratory patterns. It has a role in the region's economy with mining, salt and brine shrimp harvesting. Furthermore, the increasing urbanization and industrial development of the surrounding areas had added another level of complexity to the lake ecology with increased risk of pollution and contamination to the lake from human activity. In recent years increased interest in microorganisms from hypersaline environments has led to the discovery of new archaea and bacteria genera in the GSL. Despite those findings the GSL remains poorly studied. No serious classification of the microbial biodiversity of the lake has been undertaken since the last attempts at this type of study were performed in the 1970's and the lake microorganism population remains largely unknown and unclassified. To overcome this lack of knowledge we have started an extensive analysis of the lake microorganism population to determine the lake biodiversity and analyze the metabolic pathways involved in such an extreme environment. We have done this by utilizing nucleotide sequencing and analysis of bacteria and archaea 16S rDNA gene from DNA extracted from water and soil samples. Total genomic DNA extracted from water and lakebed soil core samples collected from the GSL north and a south arms were used as template to amplify the bacterial and archaea 16S rDNA gene by PCR amplification. The amplified 16S sequences were cloned and the nucleotide sequence determined. The sequence data were assembled using the Vector NTI program and used to search the NCBI, the Michigan State University 16S Ribosomal database and the Greengenes database for homologies. The results from this study indicated a somewhat limited microbial diversity, which to be expected considering the extreme salinity. Interestingly some organisms seemed to be broadly adaptable as they were found in multiple surrounding environments, including soil cores, while other organisms were more selective and were unique to a

particular location and sample type. More importantly some of the identified species present in the GSL have been previously isolated and described from the Yellowstone NP hot springs. This result suggests the possibility of a connection between the two locations in the past, and also a common adaptation mechanism to those extreme environments. To our knowledge this is the first study of the GSL microbial biodiversity using the 16S rDNA sequence and it may help in the understanding of the lake microbial diversity and ecology. This study will also provide some valuable information on the human impact on the lake's overall biodiversity.

6. MONITORED NATURAL ATTENUATION OF MTBE AND TBA

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Methyl tert-Butyl Ether (MTBE) and tert- Butyl Alcohol (TBA) are two of the most common groundwater pollutants in the United States. They are compounds found in gasoline and are used as an oxygenate for cleaner combustion. MTBE and TBA are most commonly introduced in to the subsurface via leaking underground fuel tanks (LUFTs). A LUFT in Ronan, Montana released these two compounds into an unconfined aquifer. Monitored natural attenuation (MNA), a process using natural biological, chemical and physical processes to reduce the presence of a compound, has been proposed as a remediation alternative to treat the MTBE and TBA plume at the Ronan site. Another LUFT site identified as Abby's Corner in Salt Lake City, Utah was also analyzed using MNA to determine if MTBE and TBA would naturally degrade using site specific organisms.

This study reported here assessed the sorption and desorption of MTBE and TBA to the soil to allow for the biodegradation of MTBE and TBA by organisms specific to the site. From the sorption procedures the coefficient of organic carbon (K_{oc}) for the Montana sediments was determined to be approximately 1.3. The higher the K_{oc} , the greater is the capacity of the solid phase (sediments or soil) to serve as a reservoir of MTBE and TBA that can then desorb from the solid phase to become available in the solution phase for microbial transformation. Desorption procedures were performed to determine the amount of MTBE and TBA removed from the sediment when water and gasoline are mixed with sediment already containing MTBE and TBA to simulate a spill of gasoline without MTBE and TBA at the site that has already experienced a spill of gasoline containing MTBE/TBA. Results of biological mineralization procedures indicated that there are organisms in the Montana sediments that can degrade MTBE and TBA associated with the sediments. From results of sorption and biodegradation experiments, we have determined that MNA is an effective way of treating MTBE and TBA at the Montana site.

7. CHANGES IN MICROBIAL COMMUNITY STRUCTURE DURING BIOSTIMULATION FOR URANIUM REDUCTION

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Former radionuclide waste ponds at the ERSP-Field Research Center in Oak Ridge, TN pose several challenges for uranium bioremediation. The site is marked by acidic conditions, high concentrations of nitrate, chlorinated solvents, and heavy metals. Above-ground treatment of groundwater, including nitrate removal via a denitrifying fluidized bed reactor (FBR) pre-conditions the groundwater for subsurface uranium immobilization. A series of re-circulating wells serve to create a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (i.e., ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 and FW-102 are the inner zones of biostimulation; and FW-024 and FW-103 are upstream and

downstream wells, respectively, which are the outer protective zones. Bacterial community composition and structure of groundwater from the wells were analyzed via clone libraries of partial SSU rRNA gene. Both qualitative and quantitative methods were used to analyze the changes in bacterial diversity and distribution. LIBSHUFF analysis was used for the comparison of bacterial community population between the different clone libraries. Bacterial community from the denitrifying FBR was different from the groundwater bacterial community, which indicated that different bacterial communities were stimulated in the two separate systems. The clone libraries of the re-circulating wells showed that over each phase of manipulation for uranium immobilization, the bacterial communities of the inner zones of biostimulation were more similar to each other and than those of the outer protective zones. The outer protective zones were more similar to the injection well. Clone libraries from FW-104 (injection), FW-101 and FW-102 showed that bacterial communities of the three wells were initially similar but developed changes through time. FW-101 and FW-102 bacterial communities developed changes in parallel, while those of FW-104 showed gradual change. These results were further compared to data generated from Unifrac analysis. Preliminary results with Unifrac analyses showed that the bacterial community in each of the wells developed changes during the bioremediation process, and the changes could be attributed to the variations along temporal, spatial, and geochemical scales. Diversity indices showed that bacterial diversity tended to increase during the initial phase of uranium bioreduction and decreased toward the end of uranium bioreduction (i.e., low U(VI) levels). As uranium levels declined, increasing *Desulfovibrio* and *Geobacter*-like sequences were detected from the clone libraries, and the *Desulfovibrio*-like sequences predominated over time. The results were further confirmed via qPCR and the results correlated with OTU distributions for *Desulfovibrio*. The results indicated that the bacterial community composition and structure changed upon stimulating for uranium bioreduction conditions, and that sequences representative of sulfate-reducers and metal-reducers were detected in wells that displayed a decline in U(VI). Further analysis is underway to determine the relationships between different functional groups and site geochemistry.

8. COEUR D'ALENE SEDIMENTS: MICROORGANISM DIVERSITY AND ZINC TOXICITY

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Abstract: Background: Mining activities in northern Idaho have left Lake Coeur d'Alene (CDA) sediments heavily enriched with toxic metals. Mean sediment concentrations of lead and zinc are 3800 and 3000 mg/kg, respectively. Under these conditions, metal-tolerant organisms may displace metal-sensitive organisms with a resulting loss in diversity. To examine this hypothesis, community analysis and bacterial isolations were conducted. **Methods:** Batch and flow reactor systems were used to isolate organisms from this metal-contaminated environment. 16S rRNA community analysis was applied to obtain clones from CDA sediment. Two isolates from CDA most closely related to *Pseudomonas* sp. LAB-06 (98%) and *Arthrobacter* sp. TSBY-20 (99%) were selected for further zinc toxicity studies, hereafter strain JM001 and JM018, respectively. To determine which aqueous zinc species presented the dominant toxicity, a thermodynamic model was used to predict zinc speciation in growth medium and used to develop hypotheses to design experiments. *A. sp.* JM018 was selected for these experiments.

A dose-structured inhibition dual-Monod form was adapted for modeling batch kinetic studies. **Results:** A variety of bacterial genera were isolated including *Ralstonia*, *Bacillus*, *Pseudomonas*, and *Arthrobacter*. Phylogenetic analysis revealed 90 clones grouped into twelve distinct phylogenetic clusters: *beta-Proteobacteria* (51/90), *Bacterioidetes* (7/90), *Acidobacteria* (6/90), *alpha-Proteobacteria* (5/90), *Flavobacteria* (5/90), *Chloroflexi* (3/90), *delta-Proteobacteria* (3/90), *gamma-Proteobacteria* (2/90), *Actinobacteria* (2/90), *Sphingobacteria* (1/90), *Chlorobi* (1/90), and *Cyanobacteria* (1/90). The major representative genera found in the sediment were *Thiobacillus* (7/90), *Azoarcus* (7/90), *Acidobacterium* (6/90), *Janthinobacterium* (5/90), and *Flavobacterium* (4/90). From isolate studies JM001 showed complete inhibition at zinc concentrations of 24 μ M and pH 7 while JM018 showed complete inhibition at 250 μ M and pH 7. Further studies with JM018 show zinc toxicity is heavily dependent on pH and zinc speciation. **Conclusions:** Kinetic expressions using dose-structured inhibition kinetics and a dual-Monod

form can model batch kinetic data of these organisms with good agreement. These findings show a great amount of genetic diversity even in highly metal contaminated sediments. Current literature predicts that the free ion activity (Zn^{2+}) will determine the toxicity, however results in this study suggest that the dominant toxic species is not the free ion, but may be a hydrolysis product ($Zn(OH)_2^0$, $Zn(OH)^+$).

9. SELENIUM SPECIATION IN SEDIMENTS, BLACKFOOT RIVER, IDAHO

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Phosphorus mining activities in the Western Phosphate Resource Area (WPRA) have released selenium (Se) that was once immobile into a dynamic weathering environment, where it has become oxidized, solubilized, and then transported throughout the surrounding ecosystem. The Blackfoot River (BFR), the main river in the watershed, and its sediments have thereby become impacted with elevated concentrations of Se. The environmental transport and bioavailability of Se is controlled by its chemical speciation. However, knowledge of the biogeochemistry and speciation of Se in streambed sediments is limited. In this study, we investigated the speciation of Se in sediment cores from the BFR using sequential extraction techniques and synchrotron-based micro-X-ray fluorescence (μ -SXRF) chemical state mapping. Four samples were analyzed from two waypoints along the BFR using both techniques. We successfully collected high resolution (20 μ m) oxidation state μ -SXRF maps of Se in sediments, which has not been done on natural sediment samples, and is currently the only technique that can probe Se speciation in natural samples without radiation damage or sample pretreatment. Selective extractions showed that most Se in the sediments is present as either 1) non-extractable Se or 2) NaOH extractable Se associated with base-soluble organic matter or tightly held on mineral surfaces. Results from μ -SXRF showed that all three defined species of Se, Se(0,II-), Se(IV), and Se(VI), were present in all four samples. Se(IV) dominated the Se speciation in samples from waypoint 04, and Se(0,II-) dominated the speciation in samples from waypoint 09. Results from both the macro- and the molecular-level methods were consistent, and in conjunction suggest that Se in Blackfoot River sediments is present as: 1) reduced species associated with recalcitrant organic matter, and 2) oxidized species tightly bound to organics and/or mineral surfaces. This information can be used to predict the bioavailability of Se in BFR sediments and the impact Se may have on the BFR ecosystem. Information on BFR sediment Se can also be used to better model and predict mobilization, biogeochemical cycling, and bioavailability of Se in other streambed sediment environments.

10. DETERMINATION OF THE BIOAVAILABLE FORMS OF COPPER AND CADMIUM

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Conceptual models such as the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM) were developed to describe bioavailability and toxicity in terms of the activity of free metal ions (M^{2+}) in aquatic systems, with the assumption that metal complexes are not bioavailable. These models are based on thermodynamic equilibrium association of the free metal ion with water-soluble inorganic and organic ligands, and ligands on cell surfaces. The adsorption of the metal to the organism surface is exclusively a function of the free metal ion in solution that can be described via adsorption isotherms. Adsorption of the free ion to the cell is a prerequisite for biological uptake and response.

Experiments were designed to evaluate the relative surface adsorption and uptake of Cd and Cu in solution as the free ion (Cd^{2+} , Cu^{2+}), as negative ($Cd[citrate]^-$, $Cu[citrate]^-$), neutral ($CdSO_4^0$, $CuSO_4^0$), and positive ($CdCl^+$, $Cu[acetate]^+$) charged complexes by bacteria. The test organism was the *Pseudomonas putida* Corvallis isolate with a *luxAB-npt* reporter cassette inserted in the Bfr mutant lacking the *bfr* gene, which encodes for the alpha subunit of the iron storage bacterioferritin protein. Batch sorption studies were conducted using solutions with variable activities of the free metal ion and metal complexes, as determined by geochemical modeling. At the end of the 1 hr sorption study, cells were rinsed with a

series of solutions to remove metals associated with the surface of the cell (5 mM CaCl₂), incorporated into the cell outer membrane (20 mM EDTA), and taken-up into the cell (nitric acid). Relationships between solution equilibrium free ion activity and the amount of Cu or Cd associated with the different phases of the cell were modeled using the Langmuir isotherm. Sorption behavior in systems with metal complexes was compared to a system with only free metal activity.

The interaction of cadmium with the cell was controlled by an ion exchange process since 82 ± 3% of the total cadmium associated with the cell was on the surface. The relative distribution of cadmium among surface, membrane and interior was not affected by the solution activity of cadmium. The majority of the copper was not on the surface but was associated with the cell membrane and internalized, with a higher amount taken up into the cell with lower solution activities. With higher concentrations, the majority of the copper was held in the membrane.

The addition of ligands resulted in enhanced cadmium associated with the cell with sulfate and citrate. Only cadmium in the presence of chloride followed the FIAM and BLM. Copper in the presence of sulfate and citrate corresponded to the initial 5 mg/L initial concentration of copper instead of the solution activity. Only copper in the presence of acetate followed the FIAM and BLM. The distribution of copper and cadmium among the surface exchange sites, membrane bound and internalized was affected the presence of the ligands.

11. PROTEINS INVOLVED IN OXYGEN SENSING AND METABOLISM ARE IMPORTANT FOR BIOFILMS IN *shewanella oneidensis* MR-1

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Shewanella oneidensis MR-1 is a versatile facultative bacterium that can utilize a variety of electron donors and acceptors, including heavy metals. Understanding the physiological responses of MR-1 to environmental stresses is important in assessing the potential impact of such perturbations on metal-reducing activity and provides insight into how metabolically diverse bacteria sense and respond to different energy sources. An improved understanding of microbial stress and survival will enable enhanced decision-making strategies regarding bioremediation as a plausible remedial strategy for contaminated field sites.

PAS domain proteins have been shown to be involved in oxygen sensing, and the roles of two closely related sensory box proteins, SO3389 and SO0341, were assessed in MR-1 for potential roles in mediating cellular responses to environmental conditions. Both ORFs contained PAS, PAC, GGDEF and EAL domains, and these domains have been implicated in multiple phenotypes; however, the exact physiological role(s) have not been fully established. Although SO3389 and SO0341 have similar domain architecture, the putative proteins exhibited different physiological responses with respect to environmental stimuli (e.g., oxygen). Initial experiments were conducted with LS4D, a minimal medium with lactate or lactate and fumarate. Aerobic growth rates were similar for the two mutants and wild-type, but motility assays differed. The mutant SO3389 displayed impaired motility compared to wild-type, but ΔSO0341 did not. Both SO3389 and SO0341 were affected in biofilm formation irrespective of rate of aeration. In addition, ΔSO0341 displayed a similar pellicle formation in both rich and defined medium, but ΔSO3389 was impaired in pellicle formation. The redox indicator in the medium also indicated that ΔSO3389 metabolized oxygen slower than wild-type or ΔSO0341.

The mutant ΔSO3389 lagged for approximately 40 h when transferred from aerobic to anoxic medium, but the growth rate was similar to wild-type once growth was initiated. The growth for ΔSO0341 was similar to wild-type during transition and anoxic growth. ΔSO3389 was also defective in cytochrome c content, fumarate reductase activity, and oxygen consumption. After the extended lag period in anoxic

medium ΔSO3389 grew but at a low population frequency, and these results suggested the original mutation was suppressed and was not an adaptation *per se*. The suppressed mutant did not lag in anoxic medium with lactate and fumarate and had a *c*-type cytochrome profile similar to wild-type, but biofilm formation was still defective and the regulation of fumarate reductase activity was altered. The results indicated that both SO0341 and SO3389 were involved cell staging for biofilm growth. Furthermore, the data indicated that SO3389 was a sensor protein involved in controlling aerobic versus anaerobic metabolism and might link responses to anoxia and biofilm growth. Further work is needed to elucidate the respective signal(s) and the mechanism(s) of signal transduction relate biofilm formation, oxygen sensing, and oxygen metabolism.

12. ANALYSIS OF MICROBIAL COMMUNITY STRUCTURE AT A METAL CONTAMINATED SITE

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Low level waste (LLW) sites are often contaminated with heavy metals and radionuclides, including chromium (Cr) and uranium (U). In addition, these sites commonly contain cellulosic waste in the form of paper towels, cardboard, wood, and other materials. We are investigating the role of cellulolytic and fermentative microorganisms on Cr and U mobility and the influence of biotic processes on metal and radionuclide mobility at such sites. An improved understanding of these processes is necessary if metal fate and transport is to be successfully understood and predicted.

Samples were removed from the Cold Test Pit South (CTPS), located at the Idaho National Laboratory (INL) Radioactive Waste Management Complex (RWMC), a Department of Energy (DOE) facility about 50 miles west of Idaho Falls, Idaho.

Soil cores were removed from an area of the pit containing stacked cardboard boxes and drums of cellulosic material. This site is stratified into three layers consisting of, from the top down, overlying fill soil, wood/cellulosic waste, and compacted soil from the base of the pit (clay). Samples were taken at four different depths within these layers of the test pit, each layer potentially harboring various microbial populations: the uppermost Fill layer (labeled F), the Fill/Waste interface (WF), the Wood Waste Layer (WW), and the Waste/Clay interface (WC).

Aerobic and anaerobic enrichments were established using a modified synthetic groundwater medium amended with either methyl cellulose or Whatman No. 1 filter paper as the sole carbon source. Aerobic and anaerobic isolates were obtained through direct isolation on solid, selective media and agar shakes. Four aerobic isolates, belonging to the *Pseudomonas*, *Streptomyces*, *Flavobacterium*, and *Pedobacter* genera, and three anaerobic isolates, two of which belong to the *Cellulomonas* and *Serratia* genera, were obtained, as shown by 16S rDNA sequencing.

The ability of one of the isolates (belonging to the *Pseudomonas* genus) to grow aerobically on a suite of relevant carbon sources (including cellulosic compounds, saccharides, and fermentation products) was measured by comparing initial protein concentrations to maximum protein concentrations reached during the growth cycle. Protein concentrations were measured using the Bradford colorimetric assay.

Microbial community analysis was completed at the Lawrence Berkeley National Laboratory using a Phylochip microarray method according to their protocol, and by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. Both methods revealed a significant shift in microbial community structure between the uppermost Fill layer and those below it.

Two of the isolates (belonging to *Pseudomonas* and *Pedobacter* genera) were used in anaerobic Cr(VI) and U(VI) reduction experiments. U(VI) and Cr(VI) concentrations were analyzed using a Kinetic Phosphorescence Analyzer (KPA) and the diphenylcarbazide (DPC) method, respectively. Total U and Cr

was measured using ICP-MS. Cells were grown aerobically on TSA before being washed, then re-suspended in anaerobic non-growth media amended with 1 g/L sucrose and 100 μ M of either Cr(VI) or U(VI). Both isolates were found capable of Cr(VI) reduction, while neither was able to reduce significant amounts of U(VI) over the course of the experiment.

13. DEHALORESPIRING AND IRON REDUCING BACTERIA DISTRIBUTIONS IN A CONTAMINATED AQUIFER

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Background: Biostimulation may be used as a relatively low cost treatment to enhance reductive dehalogenation of chlorinated ethenes that contaminate ground water. However, dissimilatory iron reducing bacteria (DIRB) may dominate energy transformation in biostimulated aquifers while the growth of populations of dehalorespiring bacteria is slowed or does not occur making treatment insufficient. It is conceivable that information about the population densities of dehalorespiring bacteria and DIRB in a contaminated aquifer prior to treatment may be useful in designing the treatment approach including determining whether augmentation with dehalorespiring bacteria is prudent. **Methods:** Twenty water table core samples, ~6-8 m deep, of a TCE contaminated aquifer were collected on a grid covering ~ 15 ha in a residential area near Hill Air Force Base, UT. DNA was extracted from the cores from subsamples taken within each core and qPCR analyses were done using primers designed to amplify the *vcrAB* and *tceA* genes, *Dehalococcoides*, *Desulfuromonas michiganensis*, *Geobacter*, *Rhodoferrax ferrireducens* and eubacterial 16S rDNA. **Results:** Concentrations of the targets varied widely over the site. *Dehalococcoides*, an important dechlorinating microorganism, was not found in all core samples. The concentrations of the *vcrAB* gene were quantifiable in 6 soil cores but at a low rate of occurrence (2/6 subsamples in 5 cores and 3/6 subsamples in one core). The concentration of this gene in the other 15 cores was not quantifiable. The concentration of *D. michiganensis* was below detection in the majority of core samples. The low incidence of detectable concentrations of *Dehalococcoides* and the *vcrAB* gene indicate that complete dehalogenation of TCE in response to biostimulation is uncertain. *R. ferrireducens*-like organisms were found in three soil cores but their incidence was lower than expected based on earlier evidence for the presence of these organisms from a single sample clone library. *Geobacter* incidence was also lower than expected. The bioavailable Fe(III) in the aquifer material was 1200 mg/kg. **Conclusions:** The variance in the population density for the target organisms across the site suggests that multiple samples would be required to characterize the reductive dehalogenation and iron reducing capability of the site. Detection limits for the methods used constrain our ability to determine the presence or absence of key organisms with confidence. Other methods, such as laboratory leaching column studies or field pilot studies may be necessary to confirm the dechlorination capability of the site.

14. COMPARISON ANALYSIS ON CELL SURFACES OF GRAM-NEGATIVE BACTERIA AND GRAM-POSITIVE BACTERIA BY ATOMIC FORCE MICROSCOPE AND RAMAN MICROSCOPY

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Abstract

The difference of the cell wall structures is one of the most distinguished variances between gram-positive and gram-negative bacteria. Gram-positive *Mycobacteria* KMS (M. KMS) and gram-negative *Pseudomonas Putida* KT2440 have been demonstrated great potential in bioremediation due to their capability of degradation of toxic polycyclic aromatic hydrocarbons (PAHs). The surface properties of the bacteria are considered to play important role in implementing of the interaction between the bacteria and the surrounding microenvironment. With the use of Atomic Force Microscope, an exquisitely sensitive

tool, we investigate the surface mechanical properties of both M. KMS and *P. putida* KT2440 through probing their interaction with the tip of AFM. The cell surface topographic features of the two bacteria are imaged at liquid or air at nanoscale resolution and the surface force profiles at single cell level of the both organisms are compared by evaluating the elastic properties and the outer layer structures. The cell surface of the KMS exhibits a flat and smooth appearance while *P. putida* KT2440 is covered by a net-like structure with average depth of 10 nm. The bacterial spring constant of the M.KMS was measured to be about 0.07N/m, and the probe indentation into surface of M.KMS is much shorter (12nm), compared with that of *P. putida* KT2440. The combination of surface topography and the force curve analysis implies the existence of a much thicker layer of exopolymers outside *P. putida* KT2440, compared to M. KMS, and the effect of these extracellular biopolymer on the surface adhesion and elastic properties for both microorganisms. As a contract, the interaction of bacteria M.KMS with the tip of AFM is primarily associated with a thinner layer of exopolymers and lipopolysacchride. The Raman images and spectra confirmed the existence of such exopolymeric structures on both bacterial surfaces, even different characteristic vibrational spectroscopic peaks are observed on both microorganisms.